LEVAMISOLE AVERMECTINS OR SIMILAR IN PYRROLIDONE SOLVENT

FIELD OF THE INVENTION

This invention relates to the field of veterinary pharmaceuticals and in particular to anthelmintic formulations including a combination of actives.

BACKGROUND

Anthelmintics are an important tool for farmers seeking to improve the productivity of grazing cattle. The first class of modern broad-spectrum anthelmintic was the benzimidazoles introduced in the early 1960's, followed by levamisole and morantel in the late 1960's and finally the avermectins and milbemycins in the early 1980's.

Anthelmintic	*Year of Introduction*	*Main Active's in the Group*
Benzimidazoles	Early 1960's	Thiabendazole, albendazole, fenbendazole, oxfendazole
Levamisole/Morantel	Late 1960's	Levamisole, morantel
Avermectins/Milbemycins	Early 1980's	Abamectin, ivermectin, moxidectin, doramectin, eprinomectin

Parasite resistance has developed to each group of anthelmintic since they were introduced. Resistance to benzimidazole-based drenches is widespread throughout the world. Cases have been reported that involve resistance in all three major cattle parasites species: *Ostertagia*, *Trichostrongylus* and *Cooperia*.

Resistance to levamisole/morantel based drenches is well known but is less widespread than benzimidazole resistance.

In 1995, New Zealand researchers reported a strain of the worm parasite *Cooperia* that was resistant to both ivermectin (a member of the avermectin/milbemycin group) and to oxfendazole (a benzimidazole). In 1996, reports were published of an ivermectin resistant *Cooperia* strain that was cross-resistant to doramectin and moxidectin (also members of the avermectin/milbemycin group).

To prevent and manage the problem of anthelmintic resistance farmers have relied on various number of strategies including:

- minimizing anthelmintic use by only treating at strategically important times
- alternating the type of anthelmintic used
- using combinations of anthelmintics from different groups to reduce the potential of parasites to survive the treatment.

Orally administered combinations of benzimidazole and levamisole anthelmintics are well known, and have been used for many years.

However in recent years products based on actives selected solely from the avermectin/milbemycin groups have held the most significant share of the cattle anthelmintic market due to their high efficacy against the major production limiting parasite species, *Ostertagia*. The availability of easy to apply topical pour-on formulations has further extended their market dominance.

By contrast, levamisole-based products have been used on a much more limited basis. Despite their having good efficacy against *Cooperia*, the key dose limiting parasite of the avermectin/milbemycin group.

The table below shows that while each anthelmintic group has particular limitations against certain parasites, a combination of actives selected from the avermectin/milbemycin and levamisole groups would achieve two highly important goals:

- high efficacy against the key cattle parasites
- combination potency to help prevent parasites surviving the treatment

Anthelmintic Class	*Cooperia Efficacy*	*Ostertagia Efficacy*
Levamisole	Good	Poor
Avermectin/Milbemycin	Poor	Good
Combination of both classes	Good	Good

Despite this rationale for an easy to use product combining levamisole active with an avermectin/milbemycin active combinations have been difficult to formulate.

Previous attempts included the formulation of a double active formulation including levamisole and niclosamide. This was designed to target tapeworm and roundworm. This formulation however, was unsatisfactory as exposure to water made it too viscous to use.

Further it was found the differing pH requirements of levamisole and other anthelmintics made it difficult to formulate a stable product.

NZ 336139 represents a recent attempt to formulate a combination avermectin/milbemycin and levamisole product.

To achieve co-existance within the formulation Nufarm relies on emulsion technology. The emulsion includes formulation including the levamisole in aqueous acidic phase and including an anthelmintic such as an avermectin or milbemycin in the lipophilic phase. A third active can be suspended in particulate form in the aqueous phase.

The disadvantage of this formulation is the need for the formulation to be shaken or agitated into an emulsion. In addition, the product is chemically complicated including 2 or 3 different phases.

The complicated nature of the formulation in NZ 336139 is due in part to the different formulation requirements of the actives. Avermectins and milbemycins being substantially insoluble in water whereas levamisole is water soluble. In addition, levamisole has previously been found to require a pH of less than about 4 for stability while avermectins and milbemycin require a pH of about 6.6.

As this will be appreciated, in addition to the stability issues topical formulations have a tendency to cause skin irritation to the animal at the site of application. Accordingly, a formulation to be acceptable for topical use it must not cause excessive skin irritation.

Accordingly, there is a need for a stable, formulation capable of stably including avermectins or milbemycins together with levamisole.

In addition, it is desirable the formulation be suitable for topical use.

OBJECT

It is an object of the present invention to provide a stable anthelmintic formulation or one that will at least provide the public with a useful choice.

STATEMENT OF INVENTION

In one aspect the invention relates to a stable formulation suitable for administration to animals including at least 2 actives wherein a first of the actives is selected from the group including the avermectins and the milbemycins and the second of said actives is levamisole, said actives being dissolved in a pyrrolidone solvent.

Preferrably the formulation may additionally include a co-solvent selected from the glycol ether group.

Preferably the avermectin or milbemycin is selected from the group including abamectin, doramectin, eprinomectin, ivermectin and moxidectin.

Preferably the pyrrolidone solvent is N-methyl pyrrolidone or 2-pyrrolidone.

More preferably the avermectin or milbemycin is present in the range of between 0.01 – 5% w/v.

Preferably levamisole is present in the range of between 1 – 30% w/v.

Preferably the formulation additionally includes at least one further medicament selected from the group comprising anthelmintics, dietary supplements, vitamins, mineral and other beneficial agents.

More preferably wherein the formulation additionally includes excipients including preservatives, stabilisers, flavorants, co solvents.

Preferably the formulation is suitable for topical, injectable or oral administration.

More preferably the formulation is suitable for topical administration.

More preferably the formulation does not cause unacceptable levels of skin irritancy when applied topically.

In a further related aspect the invention relates to a method of treating or preventing infection of cattle with *Cooperia* or *Ostertagia* by administering a formulation of the present invention.

The formulations of the present invention must be stable to be of commercial use. In this specification, a commercially acceptable anthelmintic formulation is one which is stable at room temperature for a period of at least 6 months. In conditions of accelerated testing, at 40°C, this requires the potency of the actives within the formulation to remain within specified and acceptable limits for 3 months.

Avermectins and milbemycins where used in this specification refer to a group of actives having anthelmintic activity. The avermectin group includes by way of example, avermectin, ivermectin, doramection and eprinomectin. The milbemycin group includes moxidectin.

Pyrrolidones solvents useable in this invention include, N-methyl-2-pyrrolidone, 2-pyrrolidone, 1-pyrrolidone, N-ethylene-2-pyrrolidone, 3, 3-dimethyl-2-pyrrolidone, N-ethyl-2-pyrrolidone, 5-dimethyl-2-pyrrolidone, N-ethoxy-2-pyrrolidone, and combinations thereof.

Levamisole is used in this specification includes levamisole base, levamisole phosphate together with other salts and forms.

The invention the subject of the present application is advantageous as it provides stable formulations including an avermectin or milbemycin in combination with levamisole. Further, the formulations retain each active in solution.

The formulations are monophosic and suitable to manufacture on a commercial scale. In addition, as both actives are in solution the formulations are physically stable. in that it does not separate out into separate phases either aqueous and lipophilic phases or liquid and solid phases. This enables the formulations the subject of this application to ne used without requiring agitation or shaking before use and greatly reduces the risk of differing concentrations of actives through the drum or other storage container.

In addition, as the formulation excludes water the issue of incompatible pH requirements is alleviated. Enabling the two actives to stability co-exist in a single phase.

DESCRIPTION

A large number of studies were undertaken over a 4 year period to develop a stable anthelmintic formulation combining levamisole and avermectin/milbemycin. In these studies abamectin was used as the representative avermectin/milbemycin active, whilst levamisole, in its base form, was used as the representative levamisole/morantel active.

Study 1

A number of potential formulations were prepared using a soya bean oil base and common excipients used in the preparation of topical anthelmintics.

Formulation 1		Formulation 2	
Materials	**%w/v**	**Materials**	**%w/v**
Abamectin	1	Abamectin	1
Levamisole	20	Levamisole	20
Benzyl alcohol	5	Benzyl alcohol	5
Capmul PG-8	20	Capmul PG-8	20
Isopropyl Palmitate	10	Isopropyl Myristate	10
Tween 80	2	Tween 80	2
Soya bean oil	q.v.	Soya bean oil	q.v.

Formulation 3		Formulation 4	
Materials	**%w/w**	**Materials**	**%w/w**
Abamectin	1	Abamectin	1
Benzyl alcohol	5	Levamisole	20
Capmul PG-8	20	Benzyl alcohol	5
Isopropyl Palmitate	10	Capmul PG-8	20
Tween 80	2	Isopropyl Myristate	10
Soya bean oil	q.v.	Soya bean oil	q.v.

None of these formulations were stable when tested under conditions of elevated temperature. All formulations exhibited significant degradation of the abamectin component. Animal studies also demonstrated an unexpected degree of skin irritancy with hair loss at the point of application. These results indicated that an oil-base to the product may be unsuitable both from an irritancy and stability perspective.

Study 2

A number of formulations were prepared using propylene glycol and glycol ethers, both common excipients used in veterinary drug formulation. These were then subjected to conditions of elevated temperature to determine their potential shelf stability. As a positive control for stability testing purposes a commercially available avermectin/milbemycin product, Ivomec® Plus Injection was used.

Formulations

	R20	R27	R28	R29	Ivomec®	Levipor®	Ivomec® Plus injection
Lev.base	20.0 g	20.0 g	20.0 g	20.0 g	--	20.0 g	--
Abamectin	1.0 g	1.0 g	1.0 g	1.0 g	--	--	--
Ivermectin	--	--	--	--	0.5 g	--	3.0 g
Propylene Glycol	50 g	41 g	50 g	41 g			
Benzyl alcohol	--	--	10 g	10 g			
BHT	0.2 g	0.2 g	0.2 g	0.2 g			
IPA	--	4 g	--	4 g			
*DGMEE to	100ml	100ml	100ml	100ml	* No more details		

*DGMEE: Diethylene glycol monoethyl ether (Transcutol ®)

Stability results

		0 day	5d /60°C	10d /60°C	15d /60°C	20d /60°C	25d /60°C
R20	Lev.base	100%	93.1%	92.0%	88.4%	84.9%	83.2%
	Aba	100%	86.9%	67.0%	66.5%	46.9%	34.5%
R27	Lev.base	100%	88.1%	83.6%	83.8%	83.2%	79.9%
	Aba	100%	80.7%	76.9%	67.2%	58.5%	37.6%
R28	Lev.base	100%	85.7%	82.1%	82.7%	79.5%	75.3%
	Aba	100%	84.4%	64.4%	56.5%	45.2%	39.9%
R29	Lev.base	100%	88.3%	85.6%	88.3%	85.2%	81.3%
	Aba	100%	92.2%	72.3%	63.9%	52.2%	44.5%
Ivomec ®	Ivermectin	100%	99.9%	--*	--*	--*	--*
Levipor ®	Lev.base	100%	82.0%	--*	--*	--*	--*
Ivomec ® Plus injection	Ivermectin	100%	97.9%	93.1%	91.7%	95.9% (?)	90.7%

*: solvent evaporated

In all test formulations at elevated temperatures the abamectin component degraded significantly over the period of the study. The ivermectin component of the commercially

available Ivomec® Plus formulation did not deteriorate to anywhere near the same extent as the abamectin component of the test formulations.

Whilst the levamisole component also deteriorated it did so at a much lower rate.

The study once again demonstrated the difficulty of combining the two actives and that the presence of levamisole was very problematic in preparing the combination formulation.

Study 3

A further range of formulations were prepared in which benzyl alcohol was used to solubilise the abamectin component of the formulations.

Formulations

Ingredients	Concentration (%, w/v)					
	029/0	029/1	029/2/BHT	029/3/BHT	029/4/BHA	029/5/BHA
Lev.base	20.0	20.0	20.0	20.0	20.0	20.0
Abamectin	1.0	1.0	1.0	1.0	1.0	1.0
Propylene Glycol	41.0	41.0	41.0	41.0	41.0	41.0
Benzyl Alcohol	--	15.0	15.0	15.0	15.0	15.0
Isopropyl myristate	4.0	4.0	4.0	4.0	4.0	4.0
BHT	--	--	0.2	1.0	--	--
BHA	--	--	--	--	0.2	1.0
Diethylene glycol monoethyl ether to	100ml	100ml	100ml	100ml	100ml	100ml

Stability results

		0 day	20d /60°C	25d /60°C	30d /60°C	1 Month /37°C	2 Month /37°C	3 Month /37°C
029/0	Lev.base	100%	91.0%	90.1%	88.2%	95.0%	100.9% (?)	ND
	Aba	100%	48.5%	36.3%	28.1%	91.3%	79.9%	ND
029/1	Lev.base	100%	76.3%	78.7%	75.1%	96.3%	89.4%	ND
	Aba	100%	42.3%	35.3%	31.5%	102.4%	65.8%	ND
029/2/BHT	Lev.base	100%	83.2%	74.4%	70.9%	95.4%	103.5% (?)	ND
	Aba	100%	45.3%	31.0%	31.8%	94.8%	62.8%	ND

029/3BHT	Lev.base	100%	84.1%	78.1%	70.2%	96.8%	90.8%	ND
	Aba	100%	46.2%	36.1%	32.8%	96.2%	50.2%	ND
029/4/BHA	Lev.base	100%	82.8%	73.6%	73.2%	96.9%	91.7%	ND
	Aba	100%	46.7%	34.9%	34.0%	96.5%	51.0%	ND
029/5/BHA	Lev.base	100%	85.0%	77.9%	74.5%	100.4%	94.1%	ND
	Aba	100%	47.8%	36.9%	33.0%	100.9%	53.2%	ND
Ivomec®	Iver	100%	95.0%	98.0%	101.3%	100.3%	100.3%	ND
Levipor®	Lev.base	100%	102.0%	102.9%	100.9%	104.5%	94.9%	ND

In the stability study the presence of benzyl alcohol did not have any significant effect in minimizing the rate of degradation of the abamectin component of the formulations. BHA and BHT also did not offer any advantage as stabilizing aids.

Study 4

A study was undertaken to determine whether the use of propylene glycol or glycol ethers would have any advantage in stabilizing the formulations.

Two formulations were prepared these are shown in the table below.

Formulations

	R 3	R 4
Levamisole base	20.0 g	20.0 g
Abamectin	1.0 g	1.0 g
Propylene glycol	--	40 ml
*DGBE to	100 ml	100 ml

*DGBE: Diethylene glycol n-butyl ether (Butyl carbitol®)

Stability results

		0 day	5 d/60°C	10 d/60°C	15 d/60°C	20 d/60°C
R3	Lev.base	100%	98.2%	99.0%	104.3%	100.5%
	Aba	100%	73.5%	67.3%	60.0%	52.8%
R4	Lev.base	100%	96.6%	100.6%	89.3%	95.5%
	Aba	100%	67.8%	49.6%	33.5%	33.4%

While levamisole base was relatively stable in both formulations the abamectin degraded in both formulations with the rate of degradation much more significant in the formulation that included propylene glycol. This suggested that propylene glycol was probably not beneficial in enhancing the stability of abamectin when used with DGBE.

Study 5

A study was undertaken to attempt to improve the stability of formulations that used DGBE as their base.

Formulations

	3-1	3-2	3-3
Aba	1.0 g	1.0 g	1.0 g
Leva.base	20.0 g	20.0 g	20.0 g
BHT	--	0.2 g	2.0 g
*DGBE to	100 ml	100 ml	100 ml

*DGBE: Diethylene Glycol n-butyl Ether

Stability results

		0 days	10days/60°C	20days/60°C	30days/60°C
3-1	Lev.base	100%	94.2%	96.7%	92.8%
	Aba	100%	68.8%	54.1%	40.1%
3-2	Lev.base	100%	96.8%	97.9%	91.5%
	Aba	100%	75.1%	55.9%	33.5%
3-3	Lev.base	100%	98.0%	91.1%	89.6%
	Aba	100%	73.9%	52.6%	41.1%

The study demonstrated that both BHT and BHA had no significant effect on enhancing the stability of the abamectin component of the formulation.

Study 6

Alternate formulations that used benzoic acid and/or BHT were prepared to evaluate their effects on the stability of DGBE based formulations.

Formulations

	R1	R2	R3	R4	R5	R6
Lev.base	20.0 g	20.0 g	20.0 g	20.0 g	20.0 g	20.0 g
Abamectin	1.0 g	1.0 g	1.0 g	1.0 g	1.0 g	1.0 g
BHT	--	--	--	0.2 g	0.2 g	0.2 g
Benzoic acid	--	0.05 g	0.2 g	--	0.05 g	0.2 g
*DGBE to	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

*DGBE: Diethylene Glycol n-butyl Ether

Stability results

		0 day	10d /60°C	20d /60°C	30d /60°C	1 Month /37°C	2 Month /37°C	3 Month /37°C
R1	Lev.base	100%	100.4%	98.9%	99.0%	98.7%	98.2%	98.6%
	Aba	100%	65.5%	46.1%	34.5%	88.4%	72.0%	50.6%
R2	Lev.base	100%	99.4%	98.7%	98.6%	97.9%	97.3%	96.6%
	Aba	100%	59.5%	42.3%	36.6%	71.4%	62.7%	56.6%
R3	Lev.base	100%	100.2%	103.2%	101.3%	102.4%	101.2%	102.4%
	Aba	100%	58.5%	39.1%	44.1%	85.3%	73.9%	62.8%
R4	Lev.base	100%	100.1%	98.7%	99.5%	100.2%	101.1%	100.2%
	Aba	100%	67.5%	33.7%	24.1%	93.7%	62.2%	55.2%
R5	Lev.base	100%	99.6%	99.1%	98.4%	99.2%	98.9%	99.5%
	Aba	100%	52.1%	39.0	27.7%	79.0%	61.7%	55.2%
R6	Lev.base	100%	100.1%	100.7%	99.2%	103.4%	101.2%	101.1%
	Aba	100%	53.5%	49.7%	39.9%	68.6%	62.1%	47.7%

The stability of Abamectin showed no improvement with the use of benzoic acid or BHT.

Study 7

A selection of new formulations that included other excipients with DGBE were prepared.

Formulations

	R3	R4	R5	R6
Lev.base	20.0g	15.0g	20.0g	20.0g
Lev.HCl	--	5.0g	--	--
Aba	1.0g	1.0g	1.0g	1.0g
β-CD	0.5g	--	--	--
Benzoic acid	--	--	5.0g	--
Citric acid	--	--	--	3.0g
Propylene Glycol	40ml	40ml	--	--
Glycerin Formal	30ml	30ml	--	--
Capmul MCM	--	to 100ml	--	--
DGBE	to 100ml	--	to 100ml	to 100ml

DGBE: Diethylene glycol n-butyl ether

	R7	R8	R9	R10	R11-1	R11-2	R12	R13	R14	R15
Lev.base	20.0 g	20.0 g	20.0 g	20.0 g	20.0 g	20.0 g	20.0 g	20.0 g	20.0 g	20.0 g
Aba	1.0g	1.0g	1.0g	1.0g	1.0g	1.0g	1.0g	1.0g	1.0g	1.0g
TEA	--	--	--	1.0m l	--	--	1.0m l	1.0m l	--	--

EDTA	--	--	--	--	0.01 g	--	0.01 g	0.01 g	0.01 g	0.01 g
EDTA-2Na	--	--	--	--	--	0.01 g	--	--	--	--
BHT	--	--	--	--	2.0g	2.0g	2.0g	--	2.0g	--
BHA	--	--	--	--	--	--	--	2.0g	--	2.0g
Benzoic acid	--	--	--	--	--	--	--	--	5.0g	5.0g
DGMEE	to 100 ml	--	--	to 100 ml	to 100 ml	to 100 ml	To 100 ml	to 100 ml	to 100 ml	to 100 ml
DGBE	--	to 100 ml	--	--	--	--	--	--	--	--
DPM	--	--	to 100 ml	--	--	--	--	--	--	--

TEA: Triethylamine; EDTA: Ethylenediaminetetraacetic acid; BHT: Butylated Hydroxy Tolueue; BHA: Butylated Hydroxyanisole; DGMEE: Diethylene glycol monoethyl ether; DGBE: Diethylene glycol n-butyl ether; DPM: Dipropylene glycol methyl ether

Stability results

		0 day	10days/60°C	20days/60°C	30days/60°C
R3	Lev.base	100%	99.5%	100.9%	100.9% (?)
	Aba	100%	58.7%	36.5%	37.8%
R4	Lev.base	100%	99.7%	98.8%	98.4%
	Aba	100%	58.6%	35.5%	24.0%
R5	Lev.base	100%	99.5%	90.6%	70.0%
	Aba	100%	76.2%	49.5%	42.7%
R6	Lev.base	100%	98.9%	69.5%	52.4%
	Aba	100%	70.9%	64.7%	69.4% (?)
R7	Lev.base	100%	101.1%	100.6%	100.4%
	Aba	100%	60.6%	36.5%	26.6%
R8	Lev.base	100%	99.9%	100.1%	101.0%
	Aba	100%	64.2%	52.9%	40.4%
R9	Lev.base	100%	101.4%	100.2%	98.8%
	Aba	100%	60.1%	55.4%	46.9%
R10	Lev.base	100%	94.0%	99.3%	101.7%
	Aba	100%	52.0%	37.5%	25.6%
R11-1	Lev.base	100%	101.7%	99.2%	98.3%
	Aba	100%	67.0%	40.2%	27.3%
R11-2	Lev.base	100%	106.9% (?)	100.1%	97.8%
	Aba	100%	63.3%	57.1%	38.8%
R12	Lev.base	100%	97.0%	98.8%	100.1%
	Aba	100%	53.0%	33.5%	28.3%

R13	Lev.base	100%	94.9%	99.8%	99.8%
	Aba	100%	53.3%	35.7%	28.2%
R14	Lev.base	100%	64.5%	89.4% (?)	70.6%
	Aba	100%	56.1%	38.4%	23.7%
R15	Lev.base	100%	79.7%	96.0% (?)	82.9%
	Aba	100%	67.6%	38.9%	30.2%

None of the formulations showed great promise in stabilizing the abamectin component of the formulations.

Study 8

A selection of new formulations that included other excipients with DGMEE were prepared.

Formulations

	F1	F2	F3	F4	F5	F6	F7	F8
Lev.base	20.0g	20.0g	20.0g	20.0g	20.0g	20.0g	20.0g	20.0g
Abamectin	1.0g	1.0g	1.0g	1.0g	1.0g	1.0g	1.0g	1.0g
TEA	--	1.0ml	1.0ml	--	1.0ml	1.0ml	--	--
EDTA	--	--	--	--	--	--	0.01g	0.01g
H_2O	--	--	--	10g	10g	10g	--	10g
BHT	--	--	--	--	--	--	2.0g	2.0g
BHA	--	--	--	--	--	--	--	--
Benzoic Acid	5.0g	--	5.0g	5.0g	--	5.0g	--	--
DGMEE to	100ml	100ml	100ml	100ml	100ml	100ml	100ml	100ml

TEA: Triethylamine; EDTA: Ethylenediaminetetraacetic acid; BHT: Butylated Hydroxy Toluene; BHA: Butylated Hydroxyanisole; DGMEE: Diethylene glycol monoethyl ether

Stability results

		0 day	10 days/60°C	20 days/60°C	30 days/60°C
F1	Lev.base	100%	99.6%	78.3%	63.8%
	Aba	*100%*	*69.5%*	*38.5%*	*30.3%*
F2	Lev.base	100%	100.3%	100.3%	104.6% (?)
	Aba	*100%*	*73.7%*	*50.2%*	*27.2%*
F3	Lev.base	100%	99.7%	99.9%	87.7%
	Aba	*100%*	*52.3%*	*49.8%*	*26.9%*
F4	Lev.base	100%	34.4%	9.2% (?)	8.2% (?)
	Aba	*100%*	*64.0%*	*52.8%*	*46.1%*
F5	Lev.base	100%	100.2%	97.2%	47.7%
	Aba	*100%*	*32.7%*	*No peak*	*No peak*
F6	Lev.base	100%	47.9%	40.1%	34.5%
	Aba	*100%*	*63.2%*	*55.5%*	*45.4%*

F7	Lev.base	100%	100.1%	99.0%	102.6%
	Aba	100%	72.6%	67.6%	53.9%
F8	Lev.base	100%	100.3%	99.3%	98.1%
	Aba	100%	53.6%	26.8% (?)	No peak

Once again none of the formulations showed great promise in stabilizing the abamectin component of the formulations.

Study 9

Further alternate formulations were prepared according to table below.

Formulations

	R1	R2	R3	R4	R5	R6
Lev.base	20.0g	20.0g	20.0g	20.0g	20.0g	20.0 g
Abamectin	1.0g	1.0g	1.0g	1.0g	1.0g	1.0g
Benzoic Acid	5.0g	5.0g	5.0g	10.0g	--	--
Acetic acid	--	--	--	--	2.0ml	4.0ml
BHA	--	--	2.0g	--	--	--
DGMEE to	100ml	--	--	--	--	--
DGBE to	--	100ml	100ml	100ml	100ml	100ml

BHA: Butylated Hydroxyanisole; DGMEE: Diethylene glycol monoethyl ether; DGBE: Dithylene glycol n-butyl ether

Stability results

		0 day	10 days/60°C	20 days/60°C	30 days/60°C*
R1	Lev.base	100%	105.8% (?)	85.5%	79.4%
	Aba	100%	58.1%	38.2%	31.0%
R2	Lev.base	100%	98.9%	73.9%	68.4%
	Aba	100%	71.1%	44.3%	43.6%
R3	Lev.base	100%	98.5%	73.5%	61.2%
	Aba	100%	83.8%	47.7%	38.8%
R4	Lev.base	100%	90.7%	69.0%	50.6%
	Aba	100%	53.1%	48.7%	40.6%
R5	Lev.base	100%	100.0%	99.1%	100.4%
	Aba	100%	70.0%	48.5%	28.4%
R6	Lev.base	100%	99.8%	99.6%	99.3%
	Aba	100%	57.6%	52.4%	

*The temperature in oven was changed into 55°C after stored for 20days.

However none of these demonstrated great promise in stabilizing the abamectin component of the formulations.

Study 10

Example formulations were prepared according to the table below.

Formulations

	R1	R2	R3	R4	R5
Lev.base	20.0 g	20.0 g	20.0 g	20.0 g	20.0 g
Abamectin	1.0 g	1.0 g	1.0 g	1.0 g	1.0 g
Acetic acid	--	2.0 ml	4.0 ml	6.0 ml	10.0 ml
*DGBE to	100 ml	100 ml	100 ml	100 ml	100 ml

*DGBE: Diethylene glycol n-butyl ether

Stability results

		0 day	10 days/60°C	20 days/60°C	30 days/60°C
R1	Lev.base	100%	99%	97%	96%
	Aba	100%	85%	79%	58%
R2	Lev.base	100%	82%	67%	50%
	Aba	100%	76%	71%	51%
R3	Lev.base	100%	78%	60%	40%
	Aba	100%	77%	72%	53%
R4	Lev.base	100%	52%	46%	23%
	Aba	100%	88%	85%	78%
R5	Lev.base	100%	55%	46%	19%
	Aba	100%	73%	67%	55%

Formulations containing acetic acid did not improve the stability of abamectin. However, the stability of levamisole base was adversely affected to a significant extent.

Study 11

A trial was carried out to determine whether the addition of varying levels of N-Methyl-2-Pyrollidone (Pharmasolv) to DGBE would enhance stability. All the formulations were kept at 60°C and were analysed to assess the extent of degradation after 7, 14 and 30 days.

Formulations

	G1	G2	G3	G4	G5
Lev.base	20.0% w.v.	20.0% w/v	20.0% w/v	20.0% w/v	20.0% w/v
Abamectin	1.15% w/v	1.15% w/v	1.15% w/v	1.15% w/v	1.15% w/v
DGBE	-	25% w/v	40% w/v	q/v.	q.v.
N-Methyl-2-Pyrollidone	q.v.	q.v	q.v	25%	-

Stability Results

Form.	Initial		7 days at 60°C		14 days at 60°C		1 month at 60°	
	Abamectin	Levamisole	Abamectin	Levamisole	Abamectin	Levamisole	Abamectin	Levamisole
G1	96.12	101.43	93.04	95.55	89.57	89.75	79.13	86.95
G2	100.24	103.22	95.65	99.50	95.65	96.35	79.13	93.60
G3	103.30	102.58	93.91	97.00	87.83	95.20	66.96	92.85
G4	109.05	101.70	101.74	99.95	93.91	99.35	66.57	93.80
G5	89.42	100.32	83.48	97.80	80.00	93.30	57.39	89.55

The stability results of the solution containing both the actives in Pharmasolv demonstrated that surprisingly a pyrollidone based formulation was capable of significantly slowing the rate of degradation of both levamisole and abamectin.

To further confirm the findings of this study new batches were prepared with the formulation as specified in the following table:

Material	*Formulation*
Lev.base	20.0% w/v
Abamectin	1.15% w/v
DGBE	25% w/v
N-Methyl-2-Pyrollidone	q.v

Stability results over a twelve month period of storage at 25°C confirmed the increased stability of an abamectin/levamisole formulation containing N-Methyl-2-Pyrollidone (Pharmasolv) and DGBE.

ACTIVE	*Initial*	*6 Month*	*12 Months*
Abamectin	104.00	102.55	99.95
Levamisole	99.75	99.00	98.55

Field Studies

The formulation of the table above containing DGBE and N-methyl-2- pyrollidone was used in a slaughter study to evaluate the effectiveness of the formulation relative to formulations containing either levamisole or an avermectin or milbemycin. The results clearly demonstrated that whilst the levamisole-based formulation (Levipor®) performed poorly against *Ostertagia* and the eprinomectin-based formulation (Eprinex®) performed poorly against *Cooperia*, the abamectin/levamisole combination showed outstanding efficacy against all parasite species.

A large number of field studies on cattle of all ages have also confirmed that in contrast with a number of the other test formulations there is no skin irritation on treated animals.

Table 1: Geometric mean total worm counts for calves treated with Abamectin / levamisole pour-on, Eprinex® pour-on or Levipor® pour-on in comparision with an untreated control group.

Treatment	Control	Aba/Lev PO	Eprinex® PO	Levipor® PO
Ostertagia (adult)	11435.5[a]	4.4[b]	17.3[b]	5808.1[a]
Ostertagia (immature)	1274[a]	2.3[b]	0[b]	1317.4[a]
T. axei (adult)	996.7[a]	0[b]	0[b]	110.9[a]
T.axei (immature)	4.7[a]	0[a]	0[a]	1.9[a]
Trichostrongylus spp (mature)	744.3[a]	6.7[b]	46.4[a]	5[b]
Cooperia (adult)	15948.8[a]	1.9[b]	2155.8[a]	5.9[b]
Cooperia (immature)	1598.7[a]	1.9[b]	5.7[b]	1.9[b]
Oesophagostomum (mature)	2.5[a]	0[a]	0[a]	0[a]
Trichuris (mature)	35.4[a]	0[b]	0[b]	0[b]

[a] means within the same row with different superscripts are significantly different at $p<0.05$

Table 2: Treatment efficacies based on group geometric mean total worm counts.

Treatment	Aba/Lev PO	Eprinex® PO	Levipor® PO
Ostertagia (adult)	>99.9%	99.8%	49.2%
Ostertagia (immature)	99.8%	>99.9%	0%
T. axei (adult)	>99.9%	>99.9%	80.1%
T.axei (immature)	>99.9%	>99.9%	>99.9%
Trichostrongylus spp (mature)	99.1%	93.7%	99.3%
Cooperia (adult)	>99.9%	86.5%	>99.9%

Cooperia (immature)	99.8%	99.6%	99.9%
Oesophagostomum (mature)	>99.9%	>99.9%	>99.9%
Trichuris (mature)	>99.9%	>99.9%	>99.9%

PREFERRED EMBODIMENTS

In the preferred embodiments the formulations of the invention there include avermectin or milbemycin in combination with levamisole and a pyrrolidone solvent. A glycol ether may additionally be included.

The following examples are provided as examples only and are in no way intended to limit the spirit or scope of the invention.

Example Formulations

The formulations of the present invention are prepared as follows:

1. Add levamisole base, avermectin/milbemycin and pyrollidone to a mixing vessel.
2. Stir at room temperature until the actives have completely dissolved.
3. Add the glycol ether, if desired, and mix well.
4. Add the pyrolidone to volume and continue mixing until a clear solution is obtained.

Topical Formulations

1. Examples of topically applied formulations of the invention include:

Formulation 1.1

Ingredient	*% w/v*
Abamectin	1%
Levamisole Base	20%
n-methyl pyrrolidone	q.v.

Formulation 1.2

Ingredient	*% w/v*
Ivermectin	0.5%
Levamisole Base	10%

n-methyl pyrrolidone	q.v.

Formulation 1.3

Ingredient	*% w/v*
Ivermectin	0.5%
Levamisole Base	10%
DGMBE	25%
n-methyl pyrrolidone	q.v.

Formulation 1.4

Ingredient	*% w/v*
Eprinomectin	1.0%
Levamisole Base	20%
DGMBE	25%
n-methyl pyrrolidone	q.v.

2. Examples of Injectable formulations include:

Formulation 2.1

Ingredient	*% w/v*
Ivermectin	0.5%
Levamisole Phosphate	20%
2- pyrrolidone	q.v.

Formulation 2.2

Ingredient	*% w/v*
Moxidectin	0.5%
Levamisole Phosphate	20%
2 – pyrrolidone	q.v.

3. Examples of Orally administered formulations include:

Formulation 3.1

Ingredient	*% w/v*
Abamectin	0.1%
Levamisole Base	5%
n-methyl pyrrolidone	q.v.

Formulation 3.2

Ingredient	*% w/v*
Ivermectin	1%
Levamisole Base	5%

n-methyl pyrrolidone	q.v.

Formulation 3.3

Ingredient	*% w/v*
Abamectin	1%
Levamisole Base	20%
n-methyl pyrrolidone	q.v.

Formulation 3.4

Ingredient	*% w/v*
Abamectin	1%
Levamisole Base	20%
n-methyl pyrrolidone	q.v.

Formulation 3.5

Ingredient	*% w/v*
Abamectin	1%
Levamisole Base	20%
n-methyl pyrrolidone	q.v.

The rates for these formulations are generally in the order of 1ml to 5kg to 1ml per 20kg for oral administration, 1ml per 25 kg or 1ml per 50kg for administration by injection, and 1ml per 10kg or 1ml per 20kg for topical administration.

The methods of administration of the formulations are well known within the art.